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Rocket immunoelectrophoresis to assay type D staphylococcal enterotoxin

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The problem of quick and reliable interpretation of food poisoning caused by enterotoxigenic strains of staphylococci is still a pressing one. Since the nature of the disease in many cases depends not so much on the pathogenic type contributing to the infectious process as on the type of toxin produced by it, present-day diagnosis of infectious diseases should be based on identification of not only the causative agent, but also the type of toxin produced [1]. Immunoserological methods based on the antigen—antibody reaction are being used extensively to solve these problems.

The purpose of this study was to obtain type D monospecific staphylococcal antienterotoxic serum, and use it to detect D enterotoxin by means of rocket immunoelectrophoresis.

Materials and methods

Type D staphylococcal enterotoxin (DSE) was obtained by our own system which includes salting out with ammonium sulphate, ion-exchange chromatography on diethylaminoethyl cellulose, and gel-filtration on S-200 sephacryl* [2].

DSE antiserum was prepared by immunization of rabbits weighing 2-2.5 kg. A purified preparation of the toxin was mixed with complete Freund adjuvant in a 1:1 ratio, and injected subcutaneously in doses of 1, 2.5 and 7.5 μg at the points of projection of the axillary, epigenual, inguinal and popliteal lymph nodes. Subsequent injections were carried out without adjuvant, subcutaneously and intravenously, in doses of 15, 30 and 75 μg of protein, with intervals of 7 days. The activity of the serum was tested in a gel-diffusion reaction [6].

Rocket immunoelectrophoresis was carried out by C.-B Laurell's method [5]. The serum in its final 1:20 dilution was mixed with 1.25% agarose A ("Calbiochem", USA) dissolved in a 0.05 M barbital buffer with pH 8.6, and applied to 65x90x1 mm glass slides. The toxin was placed into 10 μl lunes cut in the agarose. Watman No. 1 filter paper was used as the contact bridge between the 0.075 M barbital electrode buffer (pH 8.6) and agarose. Electrophoresis was carried out at a field intensity of 15 V/cm for 4 hours; the unprecipitated proteins were eluted with 0.15 M NaCl, and the gels were stained with Coumassi* Blue R-250 ("Serva", FRG).

The protein content of the preparations was determined by M. Bradford's method [3].

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*conjectural spelling - transl.

Type A, B, C and D staphylococcal enterotoxins manufactured by "Serva" were used as reference preparations.

Results and Discussion

The test rabbits were immunized with a purified preparation of type D enterotoxin. Since the initial dose is limited by the high toxicity of this protein (the animals died at a concentration of 5-15 μg depending on sensitivity), the first injection was 1 μg of the toxin, and the dose of the injected preparation was increased gradually. The blood of the rabbits was tested a week after the completion of the immunization cycle. The serum was separated from the clot, and lyophilized. The titre in the precipitating antibodies in the gel-diffusion reaction was 1:16.

The immunological specificity of the derived serum was studied in experiments using commercial preparations of type A, B, C and D staphylococcal enterotoxins manufactured by the "Serva" firm of the Federal Republic of Germany. In these experiments, a positive reaction was observed only in the case where the type D antienterotoxic serum interacted with homologous toxin, which indicated that it was strictly type-specific (Fig. 1, see insert).

The serum was used as the source of antibodies in rocket immunoelectrophoresis. The DSE preparation was placed in the lunes in 2-fold-diminishing concentrations of 5.0, 2.5, 1.25, 0.6, 0.3 and 0.15 μg/ml. In the agarose gel with pH 8.6, the toxin had a negative charge, since its isoelectric point is equal to 7.4 [4], and moved towards the positive electrode, forming precipitation peaks varying in height with the concentration (Fig. 2, see insert).

The correlation between the concentration of the toxin (in μg/ml) and the height of the precipitation peak (mm) is depicted in Fig. 3 by a calibration curve which is the linear relation between them. The derived calibration curve enables us to determine the quantity of type D enterotoxin in the filtrate of a S. aureus culture of strain 494 which produces this toxin. The minimal DSE concentration that we managed to detect by rocket immunoelectrophoresis amounted to 0.1 μg/ml.

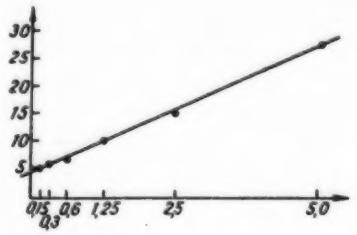

Fig. 3. Calibration curve for quantitative determination of type D staphylococcal enterotoxin (DSE) by the height of the precipitation peak after 4 hours of electrophoresis

X-axis - concentration of protein (μg/ml);
Y-axis - height of peaks (mm)

Thus, we have obtained a specific type D staphylococcal anti-enterotoxic serum which does not interact with commercial preparations of type A, B or C staphylococcal enterotoxins. The serum was used as a source of antibodies during quantitative determination of type D enterotoxin by the method of rocket immunoelectrophoresis. This is a simple method which can be used for typing staphylococcal enterotoxins and detection of toxins in food products.

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ROCKET IMMUNOELECTROPHORESIS TO ASSAY TYPE D STAPHYLOCOCCAL ENTEROTOXIN. *R. B. Naubetyarov, M. L. Beilbaeva, Yu. V. Ezepchuk*

A monospecific type D staphylococcal antienterotoxic serum has been prepared. It reacted only with the homologous toxin in gel diffusion. This serum has been used for assays of staphylococcal enterotoxin D by rocket immunoelectrophoresis.